

## CLAIMS

1. A method for identifying antibacterial agents comprising: depleting bacteria of a strain  
5 comprising a luxAB construct of  $\text{Ca}^{2+}$ , incubating the  $\text{Ca}^{2+}$  depleted bacteria with an agent  
the antibacterial effect of which shall be determined, recording the light emitted by the  
bacteria upon addition of an aldehyde, the incubation being carried out at a temperature  
which is at least  $10^{\circ}\text{C}$  higher than the temperature at which the light is emitted by the  
bacteria, preferably at least  $15^{\circ}\text{C}$  higher.  
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2. The method of claim 1, wherein said strain is a natural or mutant *Yersinia* sp. strain.
3. The method of claim 2, wherein the strain is a *Yersinia pseudotuberculosis* strain.
- 15 4. The method of claim 1, wherein the incubation temperature is about  $21^{\circ}\text{C}$  and the  
emission temperature is about  $37^{\circ}\text{C}$ , respectively.
5. A method for identifying antibacterial agents comprising:
  - providing a *Yersinia* sp. bacterial strain comprising a luxAB construct;
  - 20 - propagating the strain at room temperature in a  $\text{Ca}^{2+}$  depleting medium to obtain a  
suspension of  $\text{Ca}^{2+}$  depleted bacteria containing the luxAB construct;
  - dissolving a measured amount of a sample of an antibacterial agent candidate in  
water, a mixture of water and of an organic solvent or an organic solvent;  
organic solvent to prepare a solution of the agent;
  - 25 - combining the solution of the agent with an aliquot of the bacterial suspension to  
obtain a test suspension;
  - incubating the test suspension at a first temperature for a selected  
period of time;
  - raising the temperature of the test suspension to a second temperature;
  - 30 - continuing incubation at the second temperature for a selected period of time;
  - lowering the temperature of the test suspension to a third temperature;
  - continuing the incubation at the third temperature for a selected period of time;
  - adding n-decanal or a functionally equivalent aldehyde to the test suspension;
  - measuring light emitted from the test suspension over a period of time at the third

temperature;

- quantifying the light emitted;
- calculating an antibacterial activity based on the quantity of emitted light.

5 6. The method of claim 5, wherein said first and third temperature is from 20° C to 26° C and the second temperature is about 37° C.

7. The method of claim 5, wherein the aldehyde is decanal or a functionally equivalent aldehyde.

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8. The method of claim 5, wherein the aldehyde is added to the test suspension in form of an aqueous emulsion.

9. The method of claim 5, where in the measured amount of sample is selected to provide a  
15 concentration of the agent in the test suspension from 10 µg per mL to 100 µg per mL.

10. The method of claim 5, wherein the light emitted is less than 20 % of that emitted in an experiment in which no anti-bacterial agent had been added.

20 11. The method of claim 5, wherein the light emitted is less than 40 % of that emitted in an experiment in which no anti-bacterial agent had been added.

12. The method of claim 5, wherein the light emitted is less than 60 % of that emitted in an experiment in which no anti-bacterial agent had been added.

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13. A probe for identifying antibacterial agents comprising the *Yersinia pseudotuberculosis* strain pIB29EL.

14. A probe for identifying antibacterial agents comprising a *Yersinia pseudotuberculosis*  
30 strain selected from pIB29AL, pIB102AL, optionally also from pIB102EL.

15. A probe for identifying antibacterial agents comprising a *Yersinia pseudotuberculosis* strain selected from pIB102FL and pIB102FΔhlhL.

16. An agent capable of decreasing bacterial virulence comprising the structural element X-CO-NH-Y-Z, wherein X is aromatic or heteroaromatic carbon, Y is zero or -N=CH, and Z is unsubstituted or substituted aryl including heteroaryl.

17. An agent capable of decreasing bacterial virulence of the general formula I



wherein

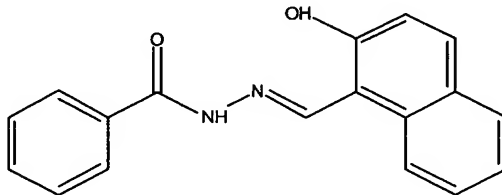
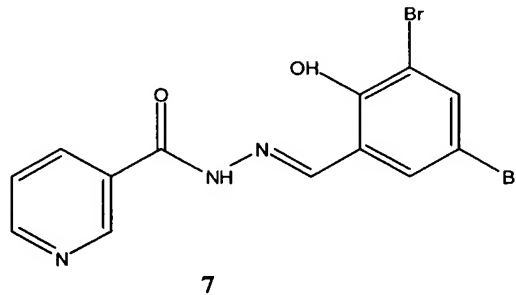
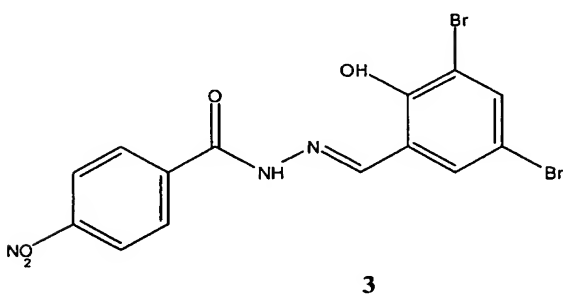
A is substituted or unsubstituted aryl or heteroaryl;

B is -X-Y, wherein X is zero or -N=CH- and Y is selected from unsubstituted aryl, unsubstituted heteroaryl, mono-, di- and tri-substituted aryl, mono-, di- and tri-substituted heteroaryl, with the proviso that, if X is -N=C-H-, Y is 2-hydroxyaryl.

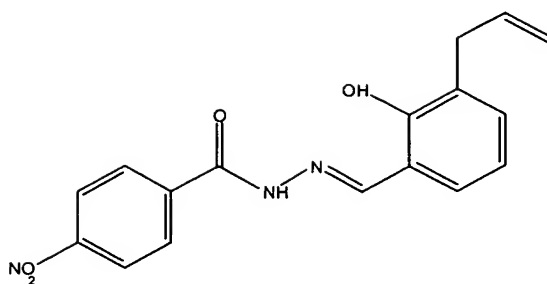
18. The agent of claim 17, wherein, if A is substituted aryl or heteroaryl, it is preferred to be mono- or disubstituted by one or more of halogen, nitro, hydroxy, alkoxy, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkenyl.

19. The agent of claim 17, wherein Y is selected from aryl and heteroaryl substituted with one or several of halogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkenyl.

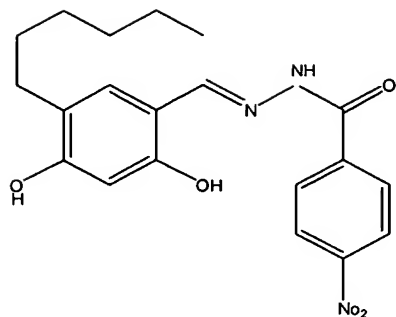
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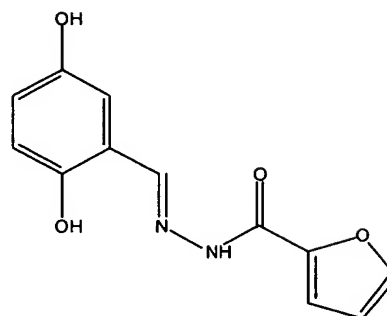
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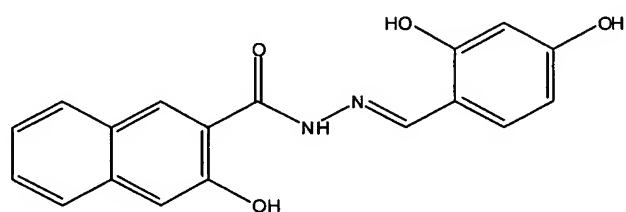
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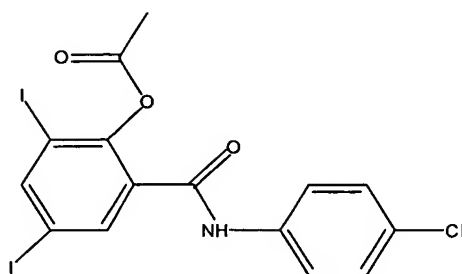


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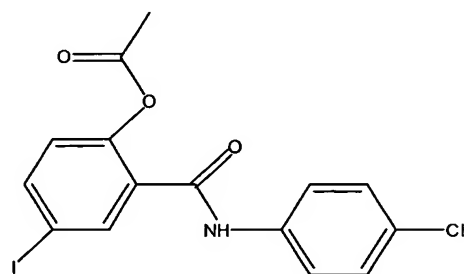


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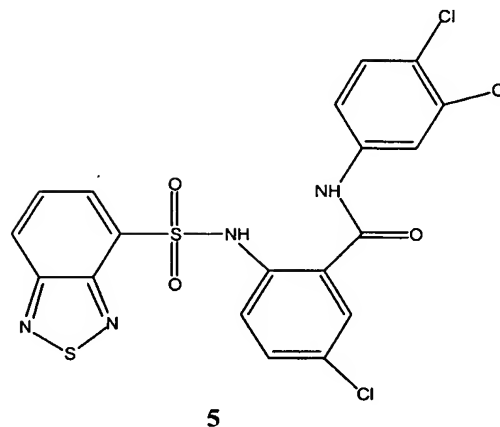
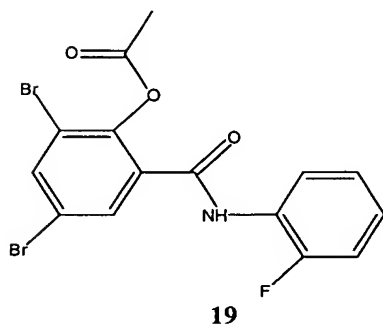
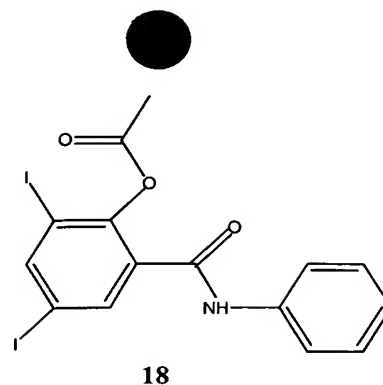
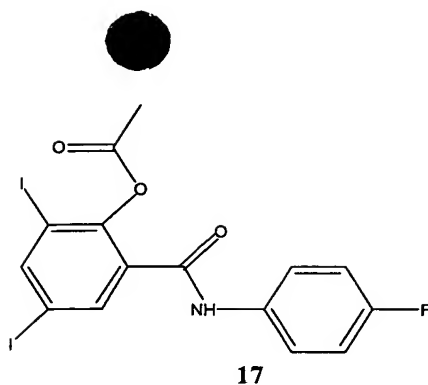


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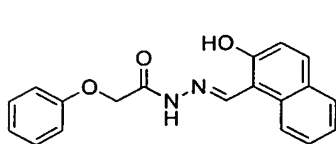
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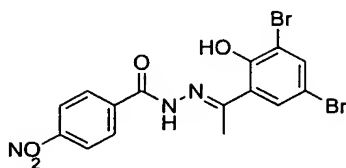
22. A method of screening for agents inhibiting virulence, the method being carried out in  
 10 absence of eukaryotic cells, comprising contacting a gram-negative bacterium culture  
 depleted in  $\text{Ca}^{2+}$ , in particular a *Yersinia* species, comprising a luxAB reporter gene  
 construct, with a potentially bacterial virulence inhibiting agent, thereby forming a test  
 suspension, manipulating the temperature of the test suspension and adding an aliphatic  
 aldehyde to make the bacterium emit light, measuring the emitted light, comparing the  
 15 amount of emitted light with the light emitted in absence of the bacterial virulence inhibiting  
 or activating agent.

23. A method of screening for agents activating bacterial virulence, the method being carried  
 out in absence of eukaryotic cells, comprising contacting a gram-negative bacterium culture  
 20 enriched in  $\text{Ca}^{2+}$ , in particular a *Yersinia* species, comprising a luxAB reporter gene  
 construct, with a potentially bacterial virulence activating agent, thereby forming a test  
 suspension, manipulating the temperature of the test suspension and adding an aliphatic  
 aldehyde to make the bacterium emit light, measuring the emitted light, comparing the  
 amount of emitted light with the light emitted in absence of the bacterial virulence inhibiting  
 25 or activating agent.

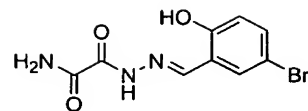
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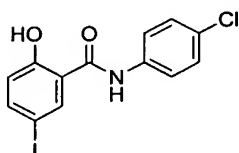
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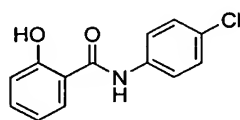
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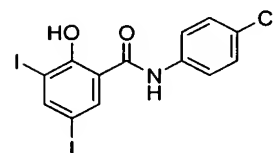
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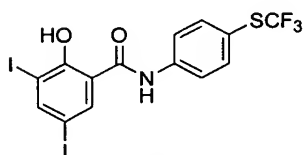
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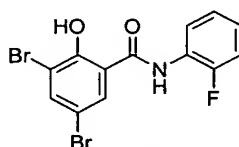
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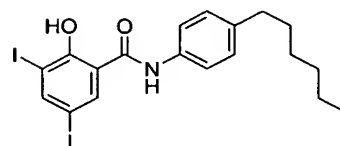
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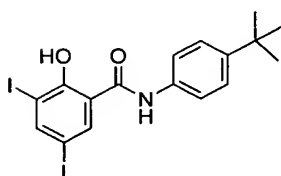
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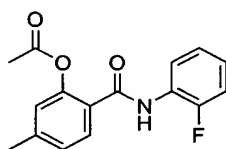
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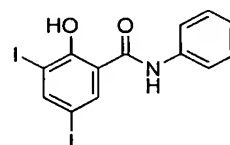
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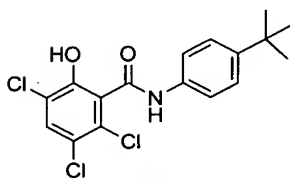
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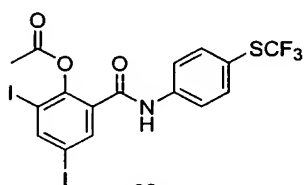
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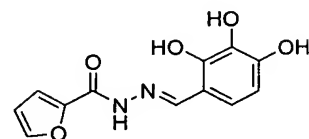
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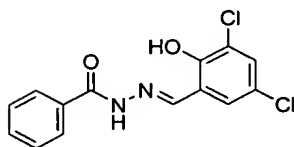
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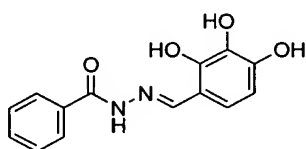
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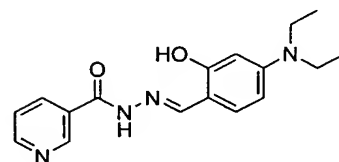
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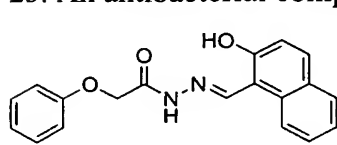


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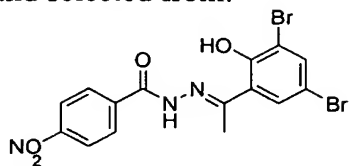


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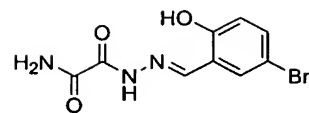
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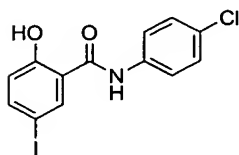
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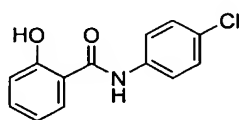
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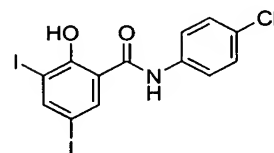
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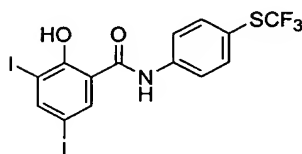
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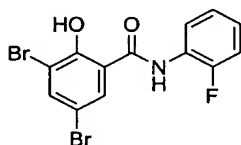
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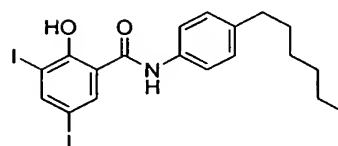
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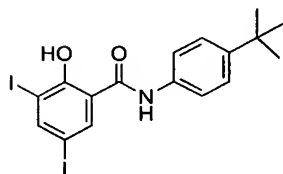
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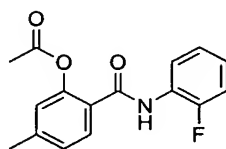
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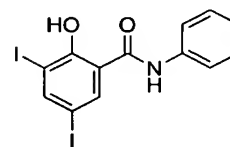
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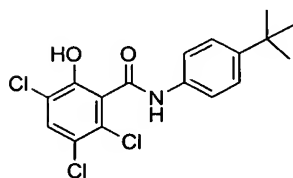
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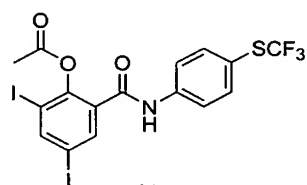
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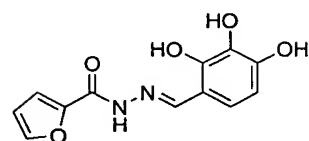
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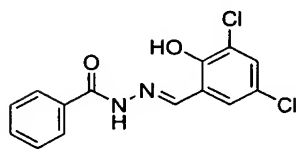
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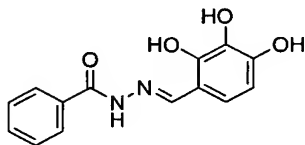
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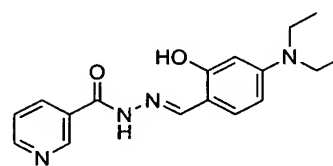
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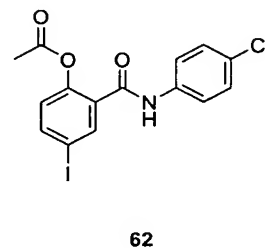
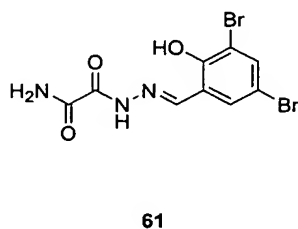
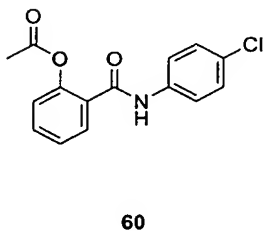
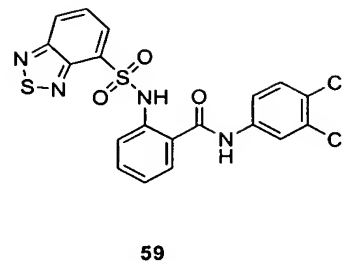
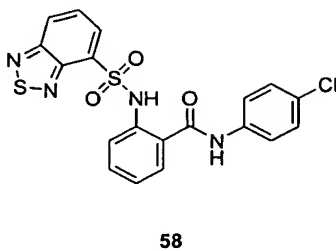
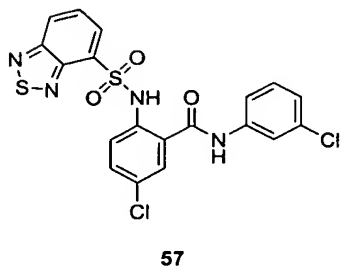


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26. An antibacterial compound selected from:



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27. An agent capable of decreasing bacterial virulence of the general formula I



wherein

10 A is substituted or unsubstituted aryl, heteroaryl, substituted or unsubstituted aryloxy or carbamyl;

B is -X-Y, wherein X is zero, -N=CH- or -CO- and Y is selected from unsubstituted aryl,

15 unsubstituted heteroaryl, mono-, di- and tri-substituted aryl, mono-, di- and tri-substituted heteroaryl, with the proviso that, if X is -N=C-H-, Y is 2-hydroxyaryl.

28. The agent of claim 27, wherein, if A is substituted aryl or heteroaryl, it is preferred to be mono- or disubstituted by one or more of halogen, nitro, hydroxy, alkoxy, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkenyl.

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29. The agent of claim 27, wherein Y is selected from aryl and heteroaryl substituted with one or several of halogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkenyl.